

AMENDMENTS TO THE SPECIFICATION

The paragraph at p. 7, lines 23-30:

Figure 2 shows the stability of ascorbic acid under culture conditions. Ascorbic acid was added to ~~mineral~~ minimal medium (2% glucose, 0.67% YNB) and incubated under standard culture conditions for 7 days. The flask of panel A was inoculated at time 0 with non-transformed *S. cerevisiae* GRF18U to an initial OD⁶⁶⁰ of 0.05, whereas the flask of panel B was kept sterile. Samples were taken at the indicated times and the ascorbic acid concentration was determined. Although the ascorbic acid was stable in this medium when growing yeast was present, it was completely degraded within 7 days in sterile medium.

The paragraph at p. 8, lines 1-7:

Figure 3 shows the endogenous ability of yeasts to convert the precursors L-galactono-1,4-lactone (Gal) or L-gulono-1,4-lactone (Gul) to ascorbic acid. Non-transformed yeast cells (*S. cerevisiae* GRF18U, W3031B and *Z. bailii*) were grown on ~~mineral~~ minimal medium (2% glucose, 0.67% YNB) in the presence of 100mM L-galactono-1,4-lactone or L-gulono-1,4-lactone, respectively, for 72 hr. (Initial OD⁶⁶⁰ was 0.05); “–” signifies that no precursor was added. While ascorbic acid was accumulated within the cell, no ascorbic acid could be detected in the culture broth.

The paragraph at p. 8, lines 9-17:

Figure 4 shows the endogenous ability of yeasts to convert L-galactose to ascorbic acid. Non-transformed *S. cerevisiae* (GRF18U and W3031B), *Z. bailii* and *K. lactis* were grown on ~~mineral~~ minimal medium (2% glucose, 0.67% YNB) starting from an OD⁶⁶⁰ of 0.05 overnight. Then, 250 mg l⁻¹ L-galactose were added and the cultures were kept under standard conditions for another 24 hr before the determination of ascorbic acid. All of these strains accumulated ascorbic acid intracellularly while no ascorbic acid was measurable in the culture broth. (It is believed the high background in *K. lactis* is due to erythroascorbic acid, naturally present in this yeast species at higher concentrations than seen in *S. cerevisiae*).

The paragraph at p. 8, lines 19-26:

Figure 5 shows the conversion of L-galactono-1,4-lactone to ascorbic acid by recombinant yeasts. *S. cerevisiae* GRF18U wt (control), or transformed with AGD or ALO, respectively, were grown on ~~minimal~~ minimal medium (2% glucose, 0.67% YNB) starting from an OD⁶⁶⁰ of 0.05 in the presence of 50 mM L-galactono-1,4-lactone (Gal) for 72 hr. While the control cells did not accumulate ascorbic acid in the culture medium, cells transformed with AGD or ALO unexpectedly accumulated considerable amounts (i.e. greater than background levels) of ascorbic acid in the culture medium. No ascorbic acid was detected in cultures without the addition of L-galactono-1,4-lactone (marked -).

The paragraph at p. 8, line 28 to p. 9, line 6:

Figure 6 shows the conversion of L-galactose to ascorbic acid by recombinant yeasts. *S. cerevisiae* GRF18U wt (control), transformed with LGDH; AGD; ALO; AGD and LGDH; ALO and LGDH; or ARA and ALO, respectively, were grown on ~~minimal~~ minimal medium (2% glucose, 0.67% YNB) starting from an OD⁶⁶⁰ of 0.05 over night. Then 250 mg l⁻¹ L-galactose were added and the cultures were kept under standard conditions for another 24 hr before the determination of ascorbic acid. The control cells or cells transformed with only LGDH did not accumulate ascorbic acid in the culture medium. Cells transformed with LGDH and either AGD or ALO, as well as cells transformed with ARA and ALO, accumulate considerable amounts (i.e. greater than background levels) of ascorbic acid in the medium.

The paragraph at p. 9, lines 8-17:

Figure 7 shows the conversion of L-galactose to ascorbic acid in a high cell density culture of recombinant yeast. *S. cerevisiae* GRF18U wt (control) or transformed with ALO, or LGDH and ALO, respectively, were grown on ~~minimal~~ minimal medium (2% glucose, 0.67% YNB) starting from an OD⁶⁶⁰ of 0.05 over night. At time 0 the cells were concentrated 10 times and 250 mg l⁻¹ L-galactose were added and the cultures were kept under standard conditions for 6 days. At the times indicated samples were taken and the ascorbic acid concentration in the culture broth was measured. While the control cells did not accumulate ascorbic acid in the culture medium, cells transformed with ALO alone or ALO and LGDH accumulated considerable amounts (i.e. greater than background levels) of ascorbic acid in the medium.